PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 15 October 1997 (15.10.97)	
International application No. PCT/GB97/00577	**Applicant's or agent's file reference** P17218/RMC
International filing date (day/month/year) 03 March 1997 (03.03.97)	**Priority date** (day/month/year) 01 March 1996 (01.03.96)
Applicant BURCHELL, Brian	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   29 September 1997 (29.09.97)

   [ ] in a notice effecting later election filed with the International Bureau on:

   _____

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

G. Bähr

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

COMMUNICATION OF
INTERNATIONAL APPLICATIONS

(PCT Article 20)

To:

United States Patent and Trademark
Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as designated Office

Date of mailing:
20 November 1997 (20.11.97)

The International Bureau transmits herewith copies of the international applications having the following international application numbers and international publication numbers:

International application no.:	International publication no.:
PCT/GB97/00577	WO97/32042

CORRECTED VERSION
VERSION CORRIGEE

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer:
J. Zahra
Telephone No.: (41-22) 338.83.38

mur-7450

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P17218/RMC	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
International application No. PCT/GB97/00577	International filing date *(day/month/year)* 03/03/1997	Priority date *(day/month/year)* 01/03/1996
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant THE UNIVERSITY COURT OF THE UNIVERSITY OF ..et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29/09/1997	03. 06. 98
Name and mailing address of the IPEA/ European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer Wieser, M Telephone No. (+49-89) 2399-8434

Form PCT/IPEA/409 (Cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB97/00577

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the [illegible] Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1,2,4-23	as originally filed			
3,3a	as received on	03/04/1998	with letter of	30/03/1998

Claims, No.:

1-14	as received on	03/04/1998	with letter of	30/03/1998

Drawings, sheets:

1/4-4/4	as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB97/00577

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-12,14
	No:	Claims	13
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-14
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	

2. Citations and explanations

see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/GB97/00577

Point V:

1. The New England Journal of Medicine, vol.333, No.18, Nov.1995, pages 1171-1175 (hereinafter referred to as document A) discloses a test for the detection of the genetic basis of the reduced expression of bilirubin UDP-Glucuronosyl-transferase 1 in Gilbert's syndrome. It is shown that the primary genetic factor contributing to Gilbert's syndrome is a 2bp insertion in the TATA box of the 5' promoter region of the gene coding for the enzyme. Document A does not explicitly disclose the use of this test in a method to improve the efficacy of drug trials.

   Thus, the subject-matter of claims 1-12 is novel in the light of the disclosure in document A (Article 33(2) PCT). The same applies to claim 14, referring to the use of specific primers which are not disclosed in the prior art.

   Claim 13, referring to a kit is anticipated by the disclosure in document A (see page 1172, methods) and does not meet the requirements of Article 33(2) PCT.

2. The subject-matter of claims 1-14 is not based on an inventive concept and does not meet the requirements of Article 33(3) PCT.

   The genetic basis of Gilbert's Syndrome, as well as a test for detecting it, is known from document A. The findings made by the authors of document A are acknowledged on page 10, lines 21-29 of the present application.

   The use of this well known test to screen samples of individuals for potential participants in a drug trial, i.e. a trial to test the efficacy of a drug in fighting Gilbert's syndrome, cannot be considered as being based on an inventive concept within the meaning of Article 33(3) PCT. In fact, no drug trial would ever be started by a skilled person without the initial step of selecting individuals from a mixed population who are indeed affected by the disease or syndrome whose response to the drug are to be tested. Any mode of proceeding which departs from this scheme would be highly illogical and counterproductive with regard to the result and evidence provided by said drug trial.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/GB97/00577

The specific primers referred to in claim 14, for use in the well known test of document A, do not seem to bring about any surprising result. Thus claim 14 is also not considered to be inventive.

Due to the benign nature of the syndrome and its prevalence in the population it may be more appropriate to consider GS as a normal genetic variant[2] exhibiting a reduced bilirubin glucuronidation capacity (which in certain situations such as fasting, illness or administration of drugs) could precipitate jaundice.

In drug trials where high levels of serum total bilirubin is detected for certain individuals, it is not clear whether this is because the individuals have Gilbert's Syndrome or if it because of an effect of the drug. Whereas presently, results are explained merely by saying that the individuals have Gilbert's Syndrome, it is suspected that in the future, it will be necessary to prove this fact.

Where a jaundiced phenotype is apparent after volunteers have been accepted for a trial and have been subjected to five days of a strict diet, no alcohol and no smoking, the jaundiced appearance giving an indication that the individuals have Gilbert's Syndrome, may cause them to be ruled out of the trials. Therefore, where approximately 250 individuals would be required for phase 1 trials and about 6000 patients for phase 3 trials, unnecessary time and effort would have been spent during the first 5 days of these trials and individuals having Gilbert's Syndrome may be ill effected.

The present invention aims to provide a method of improving the efficacy of drug trials in view of the problems mentioned above.

According to the present invention there is provided a method for improving the efficacy of drug trials, the method comprising the step of screening samples from

CLAIMS

1. A method for improving the efficacy of drug trials, the method comprising the step of screening samples from potential participants for the genetic basis of Gilbert's Syndrome and eliminating or including potential participants in a drug trial in the knowledge of them possessing or not possessing the genetic basis of Gilbert's Syndrome.

2. A method as claimed in claim 1 comprising the steps of:

   a) taking a sample from each potential participant in a drug trial,

   b) screening the samples for the genetic basis of Gilbert's Syndrome,

   c) identifying participants having the genetic basis of Gilbert's Syndrome, and

   d) proceeding with drugs trials in the knowledge of participants possessing or not possessing the genetic basis of Gilbert's Syndrome.

3 A method as claimed in claim 1 or 2 wherein the sample is chosen from blood, buccal smear or any other sample containing DNA from the potential participants.

4. A method as claimed in any of the preceding claims further comprising the step of eliminating participants having the genetic basis of Gilbert's Syndrome from a drugs trial.

5. A method as claimed in any of claims 1 to 3 wherein the method comprises the further step of selecting only participants having genetic basis for Gilbert's Syndrome for a drugs trial.

6. A method as claimed in any of claims 1 to 3 further comprising the step of interpreting the results of the drugs trial in the knowledge that certain participants have Gilbert's Syndrome.

7. A method as claimed in any of the preceding claims wherein the method comprises the steps of:

a) isolating DNA from each sample,

b) amplifying the DNA inner region indicating the genetic basis for Gilbert's Syndrome,

c) isolating amplified DNA fragments, and

d) identifying individuals having the genetic basis of Gilbert's Syndrome.

8. A method as claimed in any of the preceding claims wherein the DNA is amplified using the polymerase chain reaction (PCR) using a radioactively labelled pair of nucleotide primers.

10. A method as claimed in any of claims 7 to 9 wherein the DNA region indicating the genetic basis of Gilbert's Syndrome is the gene encoding UDP-glucuronosyltransferase (UGT).

11. A method as claimed in any of claims 7 to 10 wherein the DNA to be amplified is in an upstream promoter region of the UGT 1*1 exon 1.

12. A method as claimed in any of claims 7 to 11 wherein the DNA to be amplified includes the regions between -35 and -55 nucleotides at the 5' end of UGT 1*1 exon.

13. A kit for screening individuals participation in drug trials, the kit comprising primers for amplifying DNA in the region of the genome indicating the genetic basis of Gilbert's Syndrome.

14. Primers for use in a method as claimed in any of the preceding claims including primer pairs, AB or CD as follows:

A/B(A,5'-AAGTGAACTCCCTGCTACCTT-3',
B,5'-CCACTGGGATCAACAGTATCT-3') or
C/D (C,5'-GTCACGTGACACAGTCAAAC-3';
D 5'-TTTGCTCCTGCCAGAGGTT-3').

CORRECTED VERSION*

mur-7450

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12Q 1/68

A3

(11) International Publication Number: **WO 97/32042**

(43) International Publication Date: 4 September 1997 (04.09.97)

(21) International Application Number: PCT/GB97/00577

(22) International Filing Date: 3 March 1997 (03.03.97)

(30) Priority Data:

9604480.5	1 March 1996 (01.03.96)	GB
9605598.3	16 March 1996 (16.03.96)	GB

(71) Applicant *(for all designated States except US)*: THE UNIVERSITY COURT OF THE UNIVERSITY OF DUNDEE [GB/GB]; Tower Building, Dundee DD1 4HN (GB).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: BURCHELL, Brian [GB/GB]; 8 Dougall Street, Tayport, Fife DD6 9 JB (GB).

(74) Agent: MURGITROYD & COMPANY; 373 Scotland Street, Glasgow G5 8QA (GB).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report: 20 November 1997 (20.11.97)

(54) Title: DRUG TRIAL ASSAY SYSTEM

(57) Abstract

The invention provides a method for improving the efficacy of drug trials, the method comprising the step of screening samples from potential participants for the genetic basis of Gilbert's Syndrome and eliminating or including potential participants in a drug trial in the knowledge of them possessing or not possessing the genetic basis of Gilbert's Syndrome.

* (Referred to in PCT Gazette No. 54/1997, Section II)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 97/00577

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	N. ENGL. J. MED, vol. 333, no. 18, November 1995, pages 1171-5, XP002040437 BOSMA P ET AL: "The genetic basis of reduced expression of bilirubin UDP glucuronsyltransferase 1 in Gilbert's syndrome"	1-11
Y	see the whole document ---	1-11
Y	PHARMACOKINETICS, vol. 2, no. 3, 1992, pages 93-108, XP002040438 OWENS I ET AL: "The novel bilirubin/phenol UDP-glucuronosyltransferase UGT1 gene locus: implications for multiple familial hyperrubinemia phenotypes " see the whole document ---	1-11
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 September 1997	01. 10. 97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	**Authorized officer** Osborne, H

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/GB 97/00577

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	THE LANCET, vol. 347, 2 March 1996, pages 578-81, XP002040439 MONAGHAN G ET AL: "Genetic variation in bilirubin UDP-glucuronosyltransferase gene promoter and Gilbert's syndrome" cited in the application	1-11
Y	see the whole document	1-11
Y	WO 92 12987 A (US) 6 August 1992 see the whole document	1-11
X	GASTROENTEROLOGY, vol. 102, January 1992, pages 577-86, XP002040440 DE MORAIS S ET AL: "Decreased glucuronidation and increased bioactivation of acetaminophen in Gilbert's syndrome" cited in the application	1
Y	see abstract	2-11
X	MOLECULAR PHARMACOLOGY, vol. 43, no. 4, April 1993, pages 649-54, XP002040441 EBNER, T ET AL: "Human bilirubin UDP-gluconosyltransferase catalyzes the glucoronidation of ethinylestradiol" cited in the application	1
Y	see page 652 - page 653	2-11

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter nal Application No
PCT/GB 97/00577

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9212987 A	06-08-92	AU 1227892 A	27-08-92

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
P17218/RMC		
International application No.	International filing date (day/month/year)	(Earliest) Priority Date (day/month/year)
PCT/GB 97/00577	03/03/1997	01/03/1996

Applicant

THE UNIVERSITY COURT OF THE UNIVERSITY OF ..et al.

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing

   [ ] filed with the international application.

   [X] furnished by the applicant separately from the international application,

      [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.

   [ ] Transcribed by this Authority

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 97/00577

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	see the whole document ---	1-11
Y	PHARMACOKINETICS, vol. 2, no. 3, 1992, pages 93-108, XP002040438 OWENS I ET AL: "The novel bilirubin/phenol UDP-glucuronosyltransferase UGT1 gene locus: implications for multiple familial hyperrubinemia phenotypes " see the whole document ---	1-11
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"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 September 1997	01.10.97
Name and mailing address of the ISA: European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer: Osborne, H

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 97/00577

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	GASTROENTEROLOGY, vol. 102, January 1992, pages 577-86, XP002040440 DE MORAIS S ET AL: "Decreased glucuronidation and increased bioactivation of acetaminophen in Gilbert's syndrome" cited in the application	1
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X	MOLECULAR PHARMACOLOGY, vol. 43, no. 4, April 1993, pages 649-54, XP002040441 EBNER, T ET AL: "Human bilirubin UDP-gluconosyltransferase catalyzes the glucoronidation of ethinylestradiol" cited in the application	1
Y	see page 652 - page 653	2-11

genome sequence not known how related to hyperbillirubin & Gilbert.

suggests drug leucuronidation may be effective in drug administration.

① harvest defined population genetically

② enzyme different - not bilirubin can't use theirs as test. ethically not allowed to be done Paracetamol may work.

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 97/00577

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9212987 A	06-08-92	AU 1227892 A	27-08-92

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
P17218/RMC		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB97/00577	03/03/1997	01/03/1996

International Patent Classification (IPC) or national classification and IPC

C12Q1/68

Applicant

THE UNIVERSITY COURT OF THE UNIVERSITY OF ..et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29/09/1997	03. 06. 98
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Wieser, M Telephone No. (+49-89) 2399-8434

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB97/00577

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1,2,4-23	as originally filed			
3,3a	as received on	03/04/1998	with letter of	30/03/1998

Claims, No.:

1-14	as received on	03/04/1998	with letter of	30/03/1998

Drawings, sheets:

1/4-4/4	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-12,14
	No:	Claims	13
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-14
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	

2. Citations and explanations

see separate sheet

Point V:

1. The New England Journal of Medicine, vol.333, No.18, Nov.1995, pages 1171-1175 (hereinafter referred to as document A) discloses a test for the detection of the genetic basis of the reduced expression of bilirubin UDP-Glucuronosyl-transferase 1 in Gilbert's syndrome. It is shown that the primary genetic factor contributing to Gilbert's syndrome is a 2bp insertion in the TATA box of the 5' promoter region of the gene coding for the enzyme. Document A does not explicitly disclose the use of this test in a method to improve the efficacy of drug trials.

   Thus, the subject-matter of claims 1-12 is novel in the light of the disclosure in document A (Article 33(2) PCT). The same applies to claim 14, referring to the use of specific primers which are not disclosed in the prior art.

   Claim 13, referring to a kit is anticipated by the disclosure in document A (see page 1172, methods) and does not meet the requirements of Article 33(2) PCT.

2. The subject-matter of claims 1-14 is not based on an inventive concept and does not meet the requirements of Article 33(3) PCT.

   The genetic basis of Gilbert's Syndrome, as well as a test for detecting it, is known from document A. The findings made by the authors of document A are acknowledged on page 10, lines 21-29 of the present application.

   The use of this well known test to screen samples of individuals for potential participants in a drug trial, i.e. a trial to test the efficacy of a drug in fighting Gilbert's syndrome, cannot be considered as being based on an inventive concept within the meaning of Article 33(3) PCT. In fact, no drug trial would ever be started by a skilled person without the initial step of selecting individuals from a mixed population who are indeed affected by the disease or syndrome whose response to the drug are to be tested. Any mode of proceeding which departs from this scheme would be highly illogical and counterproductive with regard to the result and evidence provided by said drug trial.

The specific primers referred to in claim 14, for use in the well known test of document A, do not seem to bring about any surprising result. Thus claim 14 is also not considered to be inventive.

Due to the benign nature of the syndrome and its prevalence in the population it may be more appropriate to consider GS as a normal genetic variant[2] exhibiting a reduced bilirubin glucuronidation capacity (which in certain situations such as fasting, illness or administration of drugs) could precipitate jaundice.

In drug trials where high levels of serum total bilirubin is detected for certain individuals, it is not clear whether this is because the individuals have Gilbert's Syndrome or if it because of an effect of the drug. Whereas presently, results are explained merely by saying that the individuals have Gilbert's Syndrome, it is suspected that in the future, it will be necessary to prove this fact.

Where a jaundiced phenotype is apparent after volunteers have been accepted for a trial and have been subjected to five days of a strict diet, no alcohol and no smoking, the jaundiced appearance giving an indication that the individuals have Gilbert's Syndrome, may cause them to be ruled out of the trials. Therefore, where approximately 250 individuals would be required for phase 1 trials and about 6000 patients for phase 3 trials, unnecessary time and effort would have been spent during the first 5 days of these trials and individuals having Gilbert's Syndrome may be ill effected.

Bosma et al. (New England Journal of Medicine (1995) volume 333 Number 18) reported the genetic basis of Gilbert's syndrome.

The present invention aims to provide a method of improving the efficacy of drug trials in view of the problems mentioned above.

According to the present invention there is provided a method for improving the efficacy of drug trials, the method comprising the step of screening samples from

CLAIMS

1. Use of a test for detecting the genetic basis of Gilbert's Syndrome in a method to improve the efficacy of drug trials, the method comprising screening samples from potential participants for the basis of Gilbert's Syndrome and eliminating or including potential participants in a drug trial in the knowledge of them possessing or not possessing the genetic basis of Gilbert's Syndrome.

2. Use of a test as claimed in claim 1 wherein the method comprise the steps of:

   a) taking a sample from each potential participant in a drug trial,

   b) screening the samples for the genetic basis of Gilbert's Syndrome,

   c) identifying participants having the genetic basis of Gilbert's Syndrome, and

   d) proceeding with drugs trials in the knowledge of participants possessing or not possessing the genetic basis of Gilbert's Syndrome.

3 Use of a test as claimed in claim 1 or 2 wherein the sample is chosen from blood, buccal smear or any other sample containing DNA from the potential participants.

4. Use of a test as claimed in any of the preceding claims further comprising the step of eliminating participants having the genetic basis of Gilbert's

Syndrome from a drugs trial.

5. Use of a test as claimed in any of claims 1 to 3 wherein the method comprises the further step of selecting only participants having genetic basis for Gilbert's Syndrome for a drugs trial.

6. Use of a test as claimed in any of claims 1 to 3 further comprising the step of interpreting the results of the drugs trial in the knowledge that certain participants have Gilbert's Syndrome.

7. Use of a test as claimed in any of the preceding claims wherein the method comprises the steps of:

a) isolating DNA from each sample,

b) amplifying the DNA inner region indicating the genetic basis for Gilbert's Syndrome,

c) isolating amplified DNA fragments, and

d) identifying individuals having the genetic basis of Gilbert's Syndrome.

8. Use of a test as claimed in any of the preceding claims wherein the DNA is amplified using the polymerase chain reaction (PCR) using a radioactively labelled pair of nucleotide primers.

10. Use of a test as claimed in any of claims 7 to 9 wherein the DNA region indicating the genetic basis of Gilbert's Syndrome is the gene encoding UDP-glucuronosyltransferase (UGT).

11. Use of a test as claimed in any of claims 7 to 10

wherein the DNA to be amplified is in an upstream promoter region of the UGT 1*1 exon 1.

12. Use of a test as claimed in any of claims 7 to 11 wherein the DNA to be amplified includes the regions between -35 and -55 nucleotides at the 5' end of UGT 1*1 exon.

13. A kit for screening individuals participation in drug trials, the kit comprising primers for amplifying DNA in the region of the genome indicating the genetic basis of Gilbert's Syndrome.

14. Primers for use of a test as claimed in any of the preceding claims including primer pairs, AB or CD as follows:

A/B(A,5'-AAGTGAACTCCCTGCTACCTT-3',
B,5'-CCACTGGGATCAACAGTATCT-3') or
C/D (C,5'-GTCACGTGACACAGTCAAAC-3';
D 5'-TTTGCTCCTGCCAGAGGTT-3').

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(71) Applicant *(for all designated States except US)*: THE UNIVERSITY COURT OF THE UNIVERSITY OF DUNDEE [GB/GB]; Tower Building, Dundee DD1 4HN (GB).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: BURCHELL, Brian [GB/GB]; 8 Dougall Street, Tayport, Fife DD6 9 JB (GB).

(74) Agent: MURGITROYD & COMPANY; 373 Scotland Street, Glasgow G5 8QA (GB).

(54) Title: DRUG TRIAL ASSAY SYSTEM

(57) Abstract

The invention provides a method for improving the efficacy of drug trials, the method comprising the step of screening samples from potential participants for the genetic basis of Gilbert's Syndrome and eliminating or including potential participants in a drug trial in the knowledge of them possessing or not possessing the genetic basis of Gilbert's Syndrome.

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"Drug Trial Assay System"

The present invention relates to drug trials, usually carried out for or on behalf of pharmaceutical companies. More particularly the invention relates to a method for improving the efficacy of drug trials.

In the different stages of drug trials, regulatory authorities in different European countries and the FDA in the USA require extensive data to be provided in order to approve use of the drugs.

It is important that as much information as possible is available in relation to all participants who take part in drug trials, from volunteers who take part in phase 1 trials to patients involved in stage 3 clinical trials.

In particular, if certain individuals or groups of individuals have severe or abnormal reactions to drug administration, further studies involving that drug will be in jeopardy unless the reason for the reaction is realised.

The knowledge of pharmacogenetics can play an important

role in understanding the impact of drug metabolism on pharmacokinetics, role of receptor variants in drug response and in the selection of patient populations for clinical studies.

Considerable effort has been expended in attempting to identify the pharmacogenetic basis of idiosyncsatic adverse drug reactions, particularly hypersensitivity reactions. While there is clear evidence for pharmacogenetic influence on susceptibility to hypersensitivity reactions, necessary and sufficient pharamacogenetic defects have not been identified.

The clinical implications of genetic polymorphism in drug metabolism have been studied extensively (See Tucker GT (1994) Journal Pharamacology **46** pages 417-424).

Gilbert's Syndrome (GS) is a benign unconjugated hyperbilirubinaemia occurring in the absence of structural liver disease and overt haemolysis and characterized by episodes of mild intermittent jaundice. It is part of a spectrum of familial unconjugated hyperbilirubinaemias including the more severe Crigler-Najjar (CN) syndromes (types 1 and 2). GS is the most common inherited disorder of hepatic bilirubin metabolism occurring in 2-12% of the population and is often detected in adulthood through routine screening blood tests or the fasting associated with surgery/intercurrent illness which unmasks the hyperbilirubinaemia[1-3]. The most consistent feature in GS is a deficiency in bilirubin glucuronidation but altered metabolism of drugs has also been reported[3-5]. Altered rates of bilirubin production, hepatic haem production and altered hepatic uptake of bilirubin have been reported in some GS patients[2].

Due to the benign nature of the syndrome and its prevalence in the population it may be more appropriate to consider GS as a normal genetic variant[2] exhibiting a reduced bilirubin glucuronidation capacity (which in certain situations such as fasting, illness or administration of drugs) could precipitate jaundice.

In drug trials where high levels of serum total bilirubin is detected for certain individuals, it is not clear whether this is because the individuals have Gilbert's Syndrome or if it because of an effect of the drug. Whereas presently, results are explained merely by saying that the individuals have Gilbert's Syndrome, it is suspected that in the future, it will be necessary to prove this fact.

Where a jaundiced phenotype is apparent after volunteers have been accepted for a trial and have been subjected to five days of a strict diet, no alcohol and no smoking, the jaundiced appearance giving an indication that the individuals have Gilbert's Syndrome, may cause them to be ruled out of the trials. Therefore, where approximately 250 individuals would be required for phase 1 trials and about 6000 patients for phase 3 trials, unnecessary time and effort would have been spent during the first 5 days of these trials and individuals having Gilbert's Syndrome may be ill effected.

The present invention aims to provide a method of improving the efficacy of drug trials in view of the problems mentioned above.

According to the present invention there is provided a method for improving the efficacy of drug trials, the method comprising the step of screening samples from

individuals for the genetic basis of Gilbert's Syndrome.

In a prefered embodiment of the invention the method comprises the steps taking a sample from each potential participant in a drug trial, screeing the samples for the genetic basis of Gilbert's Syndrome, identifying participants having the genetic basis of Gilbert's Syndrome.

The sample may comprise blood, a buccal smear or any other sample containing DNA from the individual to be tested.

In one embodiment the method comprises the further step of eliminating participants having the genetic basis of Gilbert's Syndrome from the drug trial.

In an alternative embodiment, the method can comprise the further step of selecting participants having the genetic basis of Gilbert's syndrome and eliminating others from the drug trial.

In a further alternative the results of the drug trials can be interpreted in the knowledge that certain participants have Gilbert's Syndrome.

Preferably the method comprises the steps of isolating DNA from each sample, amplifying the DNA in a region indicating the genetic basis of Gilbert's Syndrome, isolating amplified DNA fragments by gel electrophoresis and identifying individuals having the genetic basis of Gilbert's disease.

Preferably the DNA is amplified using the polymerase chain reaction (PCR) using a radioactively labelled

pair of nucleotide primers.

The primers are designed to prime the amplification reaction at either side of an area of the genome known to be associated with Gilbert's Syndrome.

Preferably the DNA region indicating the genetic basis of Gilbert's Syndrome is the gene encoding UDP-glucuronosyltransferase (UGT).

By gene is meant, the non coding and coding regions and the upstream and downstream noncoding regions.

In a preferred embodiment the DNA to be amplified is in an upstream promoter region of the UGT1*1 exon1.

Most preferably the DNA to be amplified includes the region between -35 and -55 nucleotides at the 5' end of UGT1*1 exon.

According to the invention there are provided suitable primers for use in a PCR reaction including primer pairs;

A/B(A,5'-AAGTGAACTCCCTGCTACCTT-3',
B,5'-CCACTGGGATCAACAGTATCT-3') or
C/D (C,5'-GTCACGTGACACAGTCAAAC-3';
D 5'-TTTGCTCCTGCCAGAGGTT-3')

The invention further comprises a kit for screeing individuals for participation in drug trials, the kit comprising primers for amplifying DNA in a region of the genome indicating the genetic basis of Gilbert's Syndrome.

Using primer sequences as described herein, DNA can be amplified and analysed using among others any of the following protocols;

Protocol 1 Radioactive method

1. Extract DNA from Buccal Cells or 3ml Blood.

2. Choose primers from either side of the "TATA" box region of UGT1*1 exon1 regulatory sequence. Freshly end label one primer with [γ $^{32}\alpha$]-ATP (40 min).

3. Amplifying a small region up to 100 bp in length by PCR (2h).

4. Apply to 6% PAG denaturing gel (preparation, loading, run time, 4h).

5. Expose (-70°C) wet gel to autoradiographic film (15 min).

This method takes about 7h to complete. Polymorphisms only observed in TATA box non coding region todate.

Protocol 2
Alternative Radioactive Method: Solid Phase Minisequencing

1. Extract DNA (as above)

2. Prepare primers biotinylating one

3. Amplify DNA by PCR using primers

4. Captive biotinylated PCR products on streptavidin coated support and deactive.

5. Carry out primer extension reaction sequencing.

Protocol 3
Non-Radioactive Methods:

(a) Analysis by Single Strand Conformational Polymorphism (SSCP)

1. Extract DNA (as above).

2. Choose primers either side of the TATA Box.

3. Amplify a small region up to 100 bp in length by PCR (2H).

4. Denature and place on ice (15 min).

5. Load onto a non-denaturing PAG gel, (preparation/load/run time, 4h).

6. Stain with Ethidium bromide or silver nitrate (30 mm).

This method still takes about 7h to complete, but is potentially slightly cheaper since there is no radioactivity or autoradiography.

This method could be done on an automated DNA sequencer from stage 5, if primers are tagged with chromophores in PCR stages 2 and 3. Result would then be read automatically.

(b) Oligonucleotide Assay Hybridization

1. Extract DNA (as above).

2. Choose primers and amplify DNA by PCR up to 100 bp in length.

3. Apply DNA to plastic grids.

4. Screen bound DNA samples with specific DNA probes for TA_5, TA_6, TA_7 tagged with different coloured/fluorescent chromphores.

5. Read ouput automatically for experimental protocols.

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The basis of the invention is illustrated in the following example with reference to the accompanying figures wherein:

Figure 1 illustrates genotypes at the TATA box sequence upstream of the UGT1*1 exon 1 determined by direct sequencing and radioactive PCR.

Figure 2 illustrates serum total bilirubin (μmol/l) plotted against UGT1*1 exon 1 genotype.

Figure 3 illustrates segregation of the 7/7 genotype with elevated serum total bilirubin concentration in a family with GS.

Figure 4 illustrates the 5′ sequence of the UGT1*1 exon 1 and the position of the primers with respect to the UGT gene.

Example

We have examined the variation in the serum total bilirubin (STB) concentration in a representative group of the Eastern Scottish population (drug-free, alcohol-free non-smokers) in relation to genotype at the UDP-glucuronosyltransferase subfamily 1 (UGT1) locus. Subjects with the 77/7 genotype in this population have a significantly higher STB than those with 6/7 or 6/6 genotypes. Of 14 control subjects who underwent a 24 hour fast to establish whether they had Gilbert Syndrome (GS), only 7/77 subjects had GS. In addition, one confirmed GS patient, two recurrent jaundice patients and 9 clinically diagnosed GS patients had the 7/7 genotype. Segregation of the 7/7 genotype with elevated STB concentration has also been demonstrated in a family of 4 Gilbert members. This incidence of

the 7/7 genotype in the population is 10-13%. Here, we demonstrate a correlation between variation in the human STB concentration and genotype at a TATA sequence upstream of the UGT1*1 exon 1 and that the 7/7 genotype is diagnostic for GS.

The inheritance of GS has been described as autosomal dominant or autosomal dominant with incomplete penetrance based on biochemical analysis[6]. More recent reports have suggested that the mildly affected (Gilbert) members of families in which CN type 2 (CN-2) occurs are heterozygous for mutations in the UDI[3]-glucuronosyltransferase subfamily 1 (UGT1) gene which cause CN-2 in the homozygous state. The inheritance of GS in these families is autosomal dominant while CN-2 is autosomal recessive[7-11]. However, the incidence of CN-2 in the population is very rare and the frequency of alleles causing CN-2 would not be sufficient to explain the population incidence of GS.

An abstract by Bosma et al[12] suggested a correlation between homozygosity for a 2bp insertion in the TATA box upstream of UGT1*1 exon 1 and GS (no mutations were found in the coding sequence of the UGT1*1 gene). In this report we demonstrate that the primary genetic factor contributing to the variation in the serum total bilirubin (STB) concentration in the Eastern Scottish population is the sequence variation reported by Bosma et al[12]. In addition, we show that the 7/77 genotype is associated with GS and occurs in 10-13% of the population.

Methods

Patients and Controls

Whole blood (3ml) was collected into EDTA(K3) Vacutainer tubes (Becton Dickinson) from one confirmed

male Gilbert patient (diagnosed following a 48 hour restricted diet[13]), two female patients with recurrent jaundice/associated elevated STB (29-42 μmol/1) and 9 (1 female, 8 male) clinically diagnosed GS subjects (persistent elevation of the STB amidst normal liver function tests.) The patients were aged 22-45 years.

77 non-smoking residents selected at random from the Tayside/Fife region of Scotland (39 females aged 19-58 years, mean 32.41± 10.94; 38 males aged 23-57, means 35.58 ± 9.04) participated in this study. Whole blood (9ml) was collected 8-10am) into EDTA(K3) Vacutainer tubes (Becton Dickinson) for DNA extraction and SST Vacutainer tubes (Becton Dickinson) for biochemical investigations. The subjects had not taken any medication or alcohol in the previous 5-7 days and had fasted overnight (12 hours). 14 controls subsequently underwent further biochemical tests (following a 3 day abstinence from alcohol) before and after a 24 hour 400-calorie diet[14] to determine if they had GS. All patients/controls were fully informed of the study and gave consent for their blood to be used in this study.

Biochemistry and DNA Extraction

The following biochemical tests were performed on control blood samples; alanine aminostransferase, albumin, alkaline phosphatase, amylase, STB, cholesterol, creatinine, creatine kinase, free thyroxine, gamma-glutamyl-transferase, glucose, HDL-cholesterol, HDL-cholesterol/total cholesterol, iron, lactate dehydrogenase, percentage of saturated transferrin (PSAT), proteins, serum angiotensin converting enzyme, thyroid stimulating hormone, transferrin, triglycerides, urate, urea. 14 controls also had pre- and post-fasting (24 hour) alanine

aminostransferase, albumin, alkaline phosphatase, STB and urate measured. DNA was prepared using the Nucleon II Genomic DNA Extraction Kit (Scotlab) according to manufacturer's instructions.

Genotyping

Polymerase Chain Reaction

Primer pairs A/B (A, 5'-AAGTGAACTCCCTGCTACCTT-3'; B, 5'-CCACTGGGATCAACAGTATCT-3') or C/D (C,5'-GTCACGTGACACAGTCAAAC-3';D, 5'-TTTGCTCCTGCCAGAGGTT-3') flanking the TATA box sequence upstream of the UGTI*1 exon 1 were used to amplify fragments of 253-255bp and 98-100bp, respectively. Amplifications (50μl) were performed in 0.2mM of each deoxynucleoside triphosphate (dATP, dCTP, dGTP, dTTP), 50mM KCI, 10mM Tris.HC1 (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM $MgCl_2$, 0.25μM of each primer, 1 Unit of Taq Polymerase (Promega) and human DNA (0.25-0.5μg). The polymerase chain reaction (PCR) conditions using the Perkin-Elmer Cetus DNA Thermal Cycler were: 95°C 5 min followed by 30 cycles of 95° 30 sec, 58°C 40 sec, 72°C40 sec.

Direct Sequencing

Amplification was confirmed prior to direct sequencing by agarose gel electrophoresis. Sequencing was performed using [α-^{35}S]-dATP (NEN Dupont) with the USB Sequenase™ PCR Product Sequencing Kit according to manufacturer's instructions. Sequenced products were resolved on 6% denaturing polyacrylamide gels. The dried gels were exposed overnight to autoradiographic film prior to developing.

Radioactive PCR

Amplification was performed as above using primer pair C/D except that 2.5 pmol of primer C was radioactively 5′ end-labelled with 2.5μCi of [γ-^{32}P]-ATP (NEN Dupont) prior to amplification. Products were resolved on 6% denaturing polyacrylamide gels and the wet gels exposed to autoradiographic film (-70°C 15 min) and the autoradiographs developed.

Statistics

A t-test was used to determine if there was a significant age difference between males and females. χ^2 analysis was used to assess any difference in the distribution of the 6/6, 6/7 and 7/7 genotypes in males and females and also to determine if the 7/7 subjects from the 24 hour fasted group had STB elevated into the range diagnostic for GS[14]. An analysis of variance was performed to compare mean STB in males and females within each genotype group. A non-parametric test, the Mann-Whitney U-Wilcoxon Rank Sum W Test was used to determine whether there was a significant difference in mean STB between males and females (irrespective of genotype). Correlations and significance tests were performed for STB versus PSAT and STB versus iron. A probability (p) of < 0.05 was accepted as significant.

Results

In Figure 1 a photographic representation of the sense DNA sequences obtained by PCR/direct sequencing of DNA samples having the genotypes 6/6, 6/7 and 7/7 is shown. The common allele, $(TA)_6TAA$, is denoted by "6" while the rarer allele, $(TA)_7TAA$, is denoted by "7". Below each sequence is an overexposed photographic representation of the 98 to 100bp resolved fragments amplified using primer pair C/D which flank the TATA sequence upstream

of the UGT1*1 exon 1. The additional fragments of 99 and 101 bases are thought to be artifacts of the PCR process where there is non specified addition of an extra nucleotide to the 3' end of the amplified product[21]. Figures 1b illustrates results after testing a range of unknown individuals.

In Figure 2 males (M) and females (F) are plotted separately. Each circle/square represents the result of a single control subject. The squares indicate the 14 controls who also underwent the 24 hour restricted diet (see Methods). The filled circles/squares represent those who had a lower than normal PSAT (≤ 22%) while the half-tone circles represent those who had a higher than normal PSAT (≥ 55%). The mean STB concentrations (indicated by the horizontal lines) for males were 13.24 ± 3.88 (6/6), 13.94 ± 6.1 (6/7) including control h or 12.69 ± 3.34 excluding control h, 29 ± 14.45 (7/7) and for females were 9 ± 3.62 (6/6), 12.2 ± 3.53 (6/7), 21.6 ± 7.8 7/7). The encircled result is from control h (discussed in the text).

In Figure 3 males and females are represented by squares and circles, respectively. Filled and half-filled circles/squares indicate the genotypes 7/7 and 6/7, respectively. The numbers in parentheses below each member of the pedigree are the STB concentrations measured after a 15 hour fast and 7 day abstinence from alcohol. All family members were non smokers who were not taking any medication when the biochemical tests were performed. Elevated STB are underlined. Individual members of each generation (I or II) are denoted by the numbers 1-4 above each circle/square. Generation III have not yet been tested.

There was no significant age difference between males and females ($t = -1.38$, $p = 0.17$). Genotypes were determined initially by amplification/sequencing and later by the radioactive PCR approach. Individuals homozygous for the common allele, hetrozygous or homozygous for the rarer allele have the genotypes 6/6, 6/7 and 7/7, respective. 12 DNA samples (2 of 6/6, 3 of 6/7 and 4 of 7/7) were analysed by both methods and genotype results were identical (see Figure 1).

Genotype frequencies in male controls were 6/6 (44.74%, 6/7 (44.74%), 7/7 (10.52%) and in female controls were 6/6 (35.9%), 6/7 (51.3%), 7/7 (12.8%). There was no significant difference between the genotype proportions in the two groups ($\chi^2 = 0.6$ at 2 df, $p = 0.7$). Control h (encircled in Figure 2) had a STB which was 2.4 SD above the mean STB for that group (mean calculated including control h). The results for control h were repeatable and he is currently being investigated to exclude haemochromatosis. Comparison of mean STB in males and females revealed that females have a significantly lower concentration than males ($p = 0.031$ including control h; $p + 0.0458$ excluding control h). There was a strong correlation between genotype and mean STB concentration within the control group ($p < 0.001$) irrespective of whether control h was included and there was a significant difference in mean STB between males and females of the same genotype ($p < 0.05$) irrespective of whether control h was included (see Figure 2). All patients studied had the 7/77 genotype.

Correlations between STB/PSAT ($r = 0.4113$, $p = 0.001$)(see Figure 2) and STB/iron females ($p = 0.001$) than males ($p = 0.01$) but when control h is excluded there was no significant correlation in males.

The STB concentrations of control who underwent the 24 hour restricted diet (see Methods) are shown in Table 1. The normal fasting response is a small rise in the base-line STB (not exceeding a final concentration of 25μmol/1) most of which is unconjugated while GS patients have a lone biochemical feature a raised STB ()25μmol/1 but 〈50μmol/1) most of which is unconjugated[14]. The 6/6 and 6/7 controls had post-fasting STB of ≤23μmol/1 while all 7/77 controls were ≥31μmol/1. Other liver function tests were within acceptable ranges for the age and sex of the subjects. The 7/77 genotype correlates with a fasted STB (24 hour) within the range diagnostic for GS[14] (p 〈 0.01)(see Table 1). In addition, the 7/7 genotype segregates with elevated STB concentration in a family with 4 GS members (Figures 3).

Table 1 shows a comparison of the UGT1*1 exon 1 genotype with elevation in the serum total bilirubin after a 24 hour 400-calorie restricted diet[14].

An elevation of the fasting STB to a final concentration in the range 25-50μmol/1 is considered to be diagnostic for GS[14]. The 7/7 subject denoted by * has a fasting and non-fasting STB of 〉 50μmol/1 but this value is within a range considered by others to conform to a diagnosis of GS[7-11].

Table 1

Genotype	Sex	24 hour fast		Fasting bilirubin >25 & <50μmol/l
		Before	After	
6/6	M	8	17	NO
	M	9	19	NO
	M	12	15	NO
6/7	F	8	17	NO
	F	9	13	NO
	F	11	12	NO
	F	12	17	NO
	M	8	10	NO
	M	15	23	NO
	M	17	18	NO
7/7	F	9	34	YES
	F	12	34	YES
	M	19	31	YES
	M	62	96	NO*

Discussion

A few recent reports claim to have identified the genetic cause of GS[10-12]. Clinical diagnosis of GS is often based on a consistent midly elevated non-fasting STB (〉17 μmol/1) as the sole abnormal liver function test, intermittent jaundice or both. The diagnosis can be confirmed by elevation of the STB to 25-50μmol/1 after a 24 hour 400-calorie diet[14] or by elevation of the unconjugated bilirubin by 〉 90% within 48 hours of commencing a 400 calorie diet[13].

Sato's research group recently reported the occurrence of 7 different heteroxygous missence mutations in unrelated Gilbert patients (most of the mutations have been found in the homozygous state in affected members of CN families), however, the non-fasted STB for these patients were 〉 52μmol/1 (with the exception of one,

31μmol/l)[10,12]. These non-fasted STB concentrations already exceed the diagnostic range for GS[14], hence these patients have a more severe form of hyperbilirubinaemia than those studied in this report, while those in the Bosma et al[12] abstract had STB concentrations similar to those studied here.

The example herein shows that the variation in the STB levels after an overnight fast (and in the absence of exposure to known inducers of the UGT1*1 isoform in GS, such as alcoholic[15] and drugs[16]) a representative group of the Eastern Scottish population is primarily due to (or associated with) the TATA box sequence variation reported by Bosma et al[12]. In agreement with previous work females have a significantly lower mean STB concentration than males[17-18].

Individuals with the 7/7 genotype in the population have GS (see Table 1). One of the 7/7 controls indicated in Table 1 had a non-fasting STB similar to those reported for heterozygous carriers of CN-2 mutations[7-11] which suggests that this subject may also be a carrier of a CN-2 mutation, alternatively, the very elevated bilirubin in this patient may be due to the coexistence of Reavon's Syndrome (characterized by a collection of abnormal biochemical results which are risk factors for coronary heart disease)[19].

We have found that 10-13% of the Eastern Scottish population have the genotype associated with mild GS. None of the Gilbert subjects from the control population were aware that they had an underlying metabolic defect in glucuronidation with testifies to its benign nature. Three 7/7 controls had STB concentrations comparable to mean levels observed in heterozygotes, however, they also had a lower than

normal PSAT (≤22%)(see Figure 2). The observed correlation between STB and PSAT (p = 0.001) (Figure 2) and STB and iron (females p = 0.001 and males p = 0.01 including control h) indicates that other genetic and environmental factors affecting the serum PSAT and iron values will in turn affect the STB concentration.

From the data presented here and previous reports it seems clear that there are mild and more severe forms of GS. The milder form (fasted STB 25-50μmol/l) is either caused by (or is associated with) a homozygous 2bp insertion at the TATA sequence upstream of the UGT1*1 exon 1 (autosomal recessive inheritance) while the rarer more severe dominantly inherited forms identified to date[7-11] (non-fasted STB) 50μmol/l are due to heterozygosity for a mutation in the coding region of the UGT1*1 gene which in its homozygous state causes CN-2. The particular genetic abnormality causing GS in a patient will have implications for genetic counselling as the dominantly inherited form of two GS patients could result in offspring with CN-2, whereas the recessive form in one or both GS patients would have less serious implications. It is important to discriminate between the two forms and provide suitable genetic counselling for such couples. The rapid DNA test presented here (less than 1 day for extracted DNA) carried out in addition to biochemical tests following a 12 hour overnight fast (without prior alcohol or drug intake would permit such a diagnosis. The compliance rate for the current 24 and 48 hour restricted diet tests for GS[13-14] is debatable and hence the overnight fast has obvious advantages and only one blood sample or a buccal smear is required (for genetic and biochemical analysis) in contrast to the 2-3 blood samplings required for the 24 and 48 hour tests. This approach to GS testing would be cost effective in terms

of fewer patient return visits to clinics and in identifying couples at risk of having children with CN-2.

In addition, the recent finding of an increased bioactivation of acetominophen (a commonly used analgesic which is eliminated primarily by glucuronidation) in GS patients indicates the greater potential for drug toxicity in these patients if administered drugs which are also conjugated by UGT1 isoforms[3]. In fact, ethinylestradiol (EE2) has recently been shown to be primarily glucuronidated by the UGT1*1 isoform in man[20] and hence this could have implications for female Gilbert patients taking the oral contraceptive who are then more predisposed to developing jaundice.

The tests outlined herein have obvious implications for setting up drug trials in understanding unusual results in ruling out individuals who may be adversely affected by the drugs or in positively choosing these individuals to determine the effects of particular drugs on hyperbilirubinaemia.

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CLAIMS

1. A method for improving the efficacy of drug trials, the method comprising the step of screening samples from potential participants for the genetic basis of Gilbert's Syndrome and eliminating or including potential participants in a drug trial in the knowledge of them possessing or not possessing the genetic basis of Gilbert's Syndrome.

2. A method as claimed in claim 1 comprising the steps of:

a) taking a sample from each potential participant in a drug trial,

b) screening the samples for the genetic basis of Gilbert's Syndrome,

c) identifying participants having the genetic basis of Gilbert's Syndrome, and

d) proceeding with drugs trials in the knowledge of participants possessing or not possessing the genetic basis of Gilbert's Syndrome.

3 A method as claimed in claim 1 or 2 wherein the sample is chosen from blood, buccal smear or any other sample containing DNA from the potential participants.

4. A method as claimed in any of the preceding claims further comprising the step of eliminating participants having the genetic basis of Gilbert's Syndrome from a drugs trial.

5. A method as claimed in any of claims 1 to 3 wherein the method comprises the further step of selecting only participants having genetic basis for Gilbert's Syndrome for a drugs trial.

6. A method as claimed in any of claims 1 to 3 further comprising the step of interpreting the results of the drugs trial in the knowledge that certain participants have Gilbert's Syndrome.

7. A method as claimed in any of the preceding claims wherein the method comprises the steps of:

a) isolating DNA from each sample,

b) amplifying the DNA inner region indicating the genetic basis for Gilbert's Syndrome,

c) isolating amplified DNA fragments, and

d) identifying individuals having the genetic basis of Gilbert's Syndrome.

8. A method as claimed in any of the preceding claims wherein the DNA is amplified using the polymerase chain reaction (PCR) using a radioactively labelled pair of nucleotide primers.

10. A method as claimed in any of claims 7 to 9 wherein the DNA region indicating the genetic basis of Gilbert's Syndrome is the gene encoding UDP-glucuronosyltransferase (UGT).

11. A method as claimed in any of claims 7 to 10 wherein the DNA to be amplified is in an upstream promoter region of the UGT 1*1 exon 1.

12. A method as claimed in any of claims 7 to 11 wherein the DNA to be amplified includes the regions between -35 and -55 nucleotides at the 5′ end of UGT 1*1 exon.

13. A kit for screening individuals participation in drug trials, the kit comprising primers for amplifying DNA in the region of the genome indicating the genetic basis of Gilbert's Syndrome.

14. Primers for use in a method as claimed in any of the preceding claims including primer pairs, AB or CD as follows:

A/B(A,5′-AAGTGAACTCCCTGCTACCTT-3′,
B,5′-CCACTGGGATCAACAGTATCT-3′) or
C/D (C,5′-GTCACGTGACACAGTCAAAC-3′;
D 5′-TTTGCTCCTGCCAGAGGTT-3′).

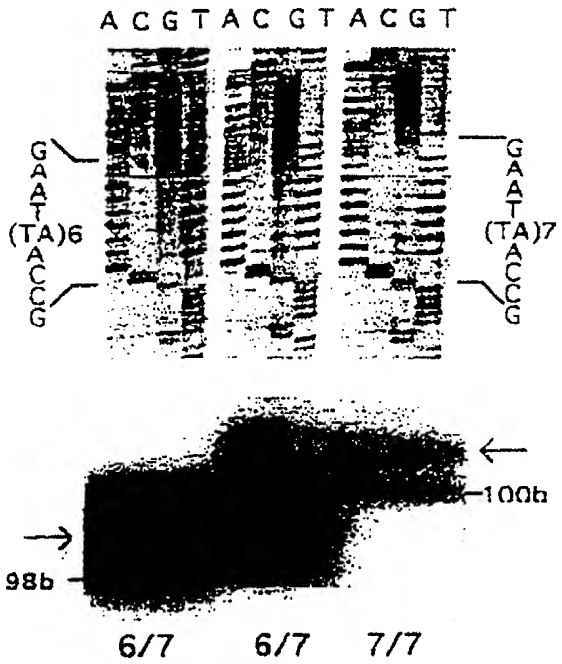

A C G T A C G T A C G T
GAAT(TA)6ACCG
GAAT(TA)7ACCG
100b
98b
6/7 6/7 7/7

Fig. 1a


G
100
TA7
TA6

Fig. 1b

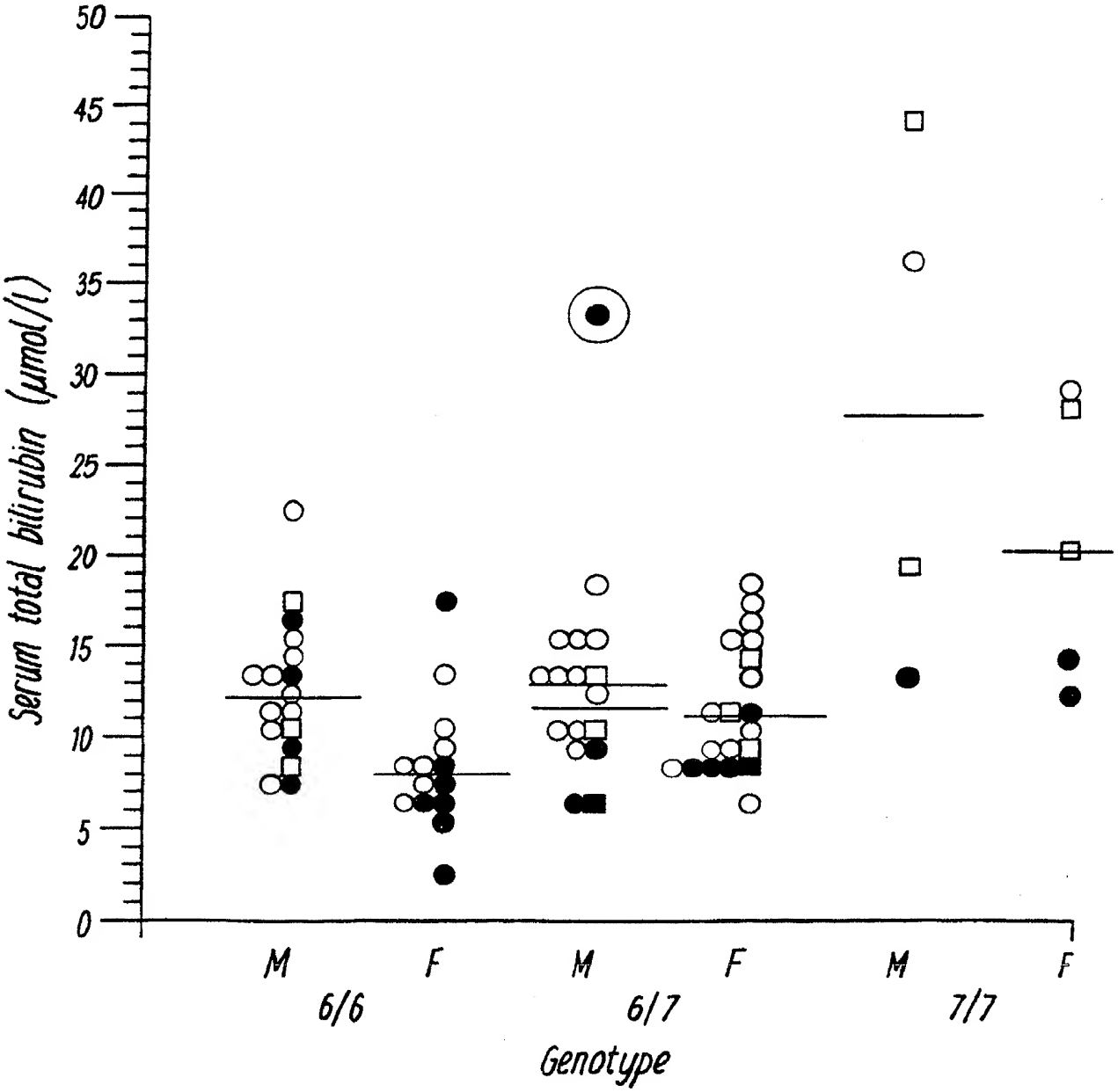
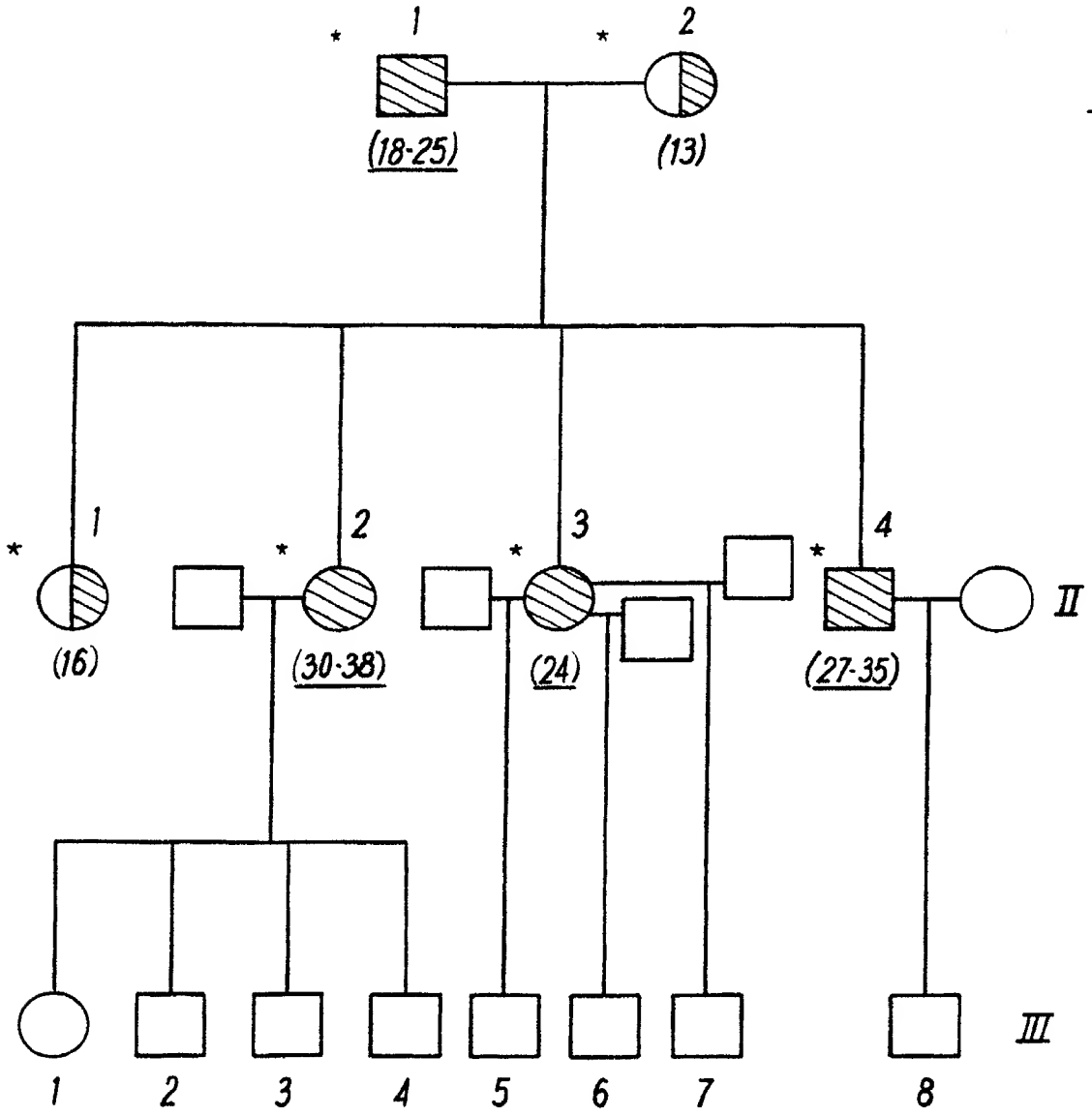

Serum total bilirubin (μmol/l)
50
45
40
35
30
25
20
15
10
5
0
M
F
M
F
M
F
6/6
6/7
7/7
Genotype

FIG. 2

Pedigree Showing Segregation of the Gilbert Phenotype with the $(TA)_7TAA/(TA)_7TAA$ Genotype.



I, II, III = generations in family * = genetic and biochemical data available

□ male ▨ ◎ homozygotes for the $(TA)_7TAA$ allele

○ female ◐ heterozygotes for the $(TA)_7TAA$ and $(TA)_6TAA$ alleles

(13) = total serum bilirubin (18-25) = elevated total serum bilirubin

FIG. 3

AP1

-611 GTGAGTCTGGCTCACCTCATGGCGCGTGGCTCGTGTGGTGGGCTCTGCTGCAGCCTCCAA

Sp1

-541 GACACCACACTGTGCTGGACTCAATAAATAATGTTGGACGAAGGAATGAAACACATGATA

-491 CAAGTGAGCAGGCAGTACCGGGGGAGCTGTGGAGTGGGCACTCTTACAGGTTTCCATGGC

-431 GAAAGCGGGGGGACAGTTGTGTTCTTTTCTTTCTAAAAGGCTTTCTAAAAAGCCTTCTGT

-371 TTAATTTCTGGAAAAGAAGCCTAACTTGTTCACTACATAGTCGTCCTTCTTCCTCTCTGG

AP3

-311 TAACACTTGTTGGTCTGTGGAAATACTAATTTAATGGATCCTGAGGTTCTGGAAGTACTT

CLBP AP1

-251 TGCTGTGTTCACTCAAGAATGTGATTTGAGTATGAAATTCCAGCCAGTTCAACTGTTGTT

-191 GCCTATTAAGAAACCTAATAAAGCTCCACCTTCTTTATCTCTGAAAGTGAACTCCCTGCT

AP1 HNF1

-131 ACCTTTGTGGACTGACAGCTTTTTATAGTCACGTGACACAGTCAAACATTAACTTGGTGT

-71 ATCGATTGGTTTTTGCCATATATATATATATAAGTAGGAGAGGGCGAACCTCTGGCAGGA

-11 GCAAAGGCGCCATGGCTGTG

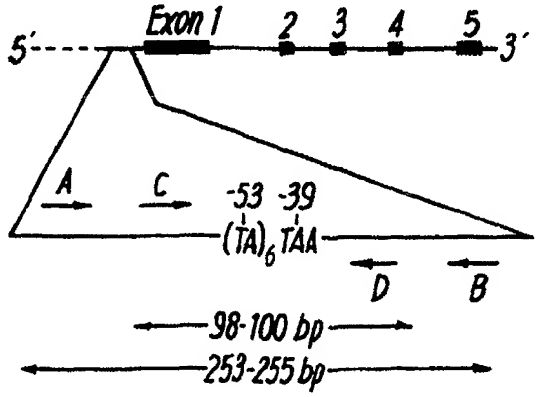


FIG. 4

MUR-7450

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
P17218/RMC		
International application No.	International filing date (day/month/year)	(Earliest) Priority Date (day/month/year)
PCT/GB 97/00577	03/03/1997	01/03/1996

Applicant

THE UNIVERSITY COURT OF THE UNIVERSITY OF ..et al.

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing

   [ ] filed with the international application.

   [X] furnished by the applicant separately from the international application,

      [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.

   [ ] Transcribed by this Authority

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/00577

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	✓N. ENGL. J. MED, vol. 333, no. 18, November 1995, pages 1171-5, XP002040437 BOSMA P ET AL: "The genetic basis of reduced expression of bilirubin UDP glucuronsyltransferase 1 in Gilbert's syndrome"	1-11
Y	see the whole document ---	1-11
Y	✓PHARMACOKINETICS, vol. 2, no. 3, 1992, pages 93-108, XP002040438 OWENS I ET AL: "The novel bilirubin/phenol UDP-glucuronosyltransferase UGT1 gene locus: implications for multiple familial hyperrubinemia phenotypes " see the whole document ---	1-11
	-/--	

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 September 1997	01. 10. 97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Osborne, H

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	THE LANCET, vol. 347, 2 March 1996, pages 578-81, XP002040439 MONAGHAN G ET AL: "Genetic variation in bilirubin UDP-glucuronosyltransferase gene promoter and Gilbert's syndrome" cited in the application	1-11
Y	see the whole document	1-11

Y	WO 92 12987 A (US) 6 August 1992 see the whole document	1-11

X	GASTROENTEROLOGY, vol. 102, January 1992, pages 577-86, XP002040440 DE MORAIS S ET AL: "Decreased glucuronidation and increased bioactivation of acetaminophen in Gilbert's syndrome" cited in the application	1
Y	see abstract	2-11

X	MOLECULAR PHARMACOLOGY, vol. 43, no. 4, April 1993, pages 649-54, XP002040441 EBNER, T ET AL: "Human bilirubin UDP-gluconosyltransferase catalyzes the glucoronidation of ethinylestradiol" cited in the application	1
Y	see page 652 - page 653	2-11

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 97/00577

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9212987 A	06-08-92	AU 1227892 A	27-08-92